

**WHAT IS CLAIMED IS:**

1                   1.     A method of extracting structural information from a NMR data set for  
2 a selected macromolecule in an intact biological compartment wherein said selected  
3 macromolecule is labeled with an NMR-detectable nucleus, such that said nucleus is present  
4 in said macromolecule in an amount greater than is naturally abundant in said  
5 macromolecule, said method comprising:

6           (a) contacting said cell with radio frequency energy, thereby producing an excited  
7           NMR-detectable nucleus;

8           (b) collecting radio frequency data from said excited NMR-detectable nucleus,  
9           thereby producing said NMR data set, and

10          (c) analyzing said data set to extract said structural information for said selected  
11          macromolecule from said data set.

1                   2.     The method according to claim 1, wherein said selected  
2 macromolecule is overexpressed in said biological compartment.

1                   3.     The method according to claim 1, wherein said NMR-detectable  
2 nucleus is present in an amount detectable by NMR of said biological compartment.

1                   4.     The method according to claim 1, wherein said selected  
2 macromolecule is a member selected from the group consisting of proteins, saccharides,  
3 glycoproteins, and nucleic acids.

1                   5.     The method according to claim 1, wherein said selected  
2 macromolecule is in a complex with a small molecule.

1                   6.     The method according to claim 5, wherein said small molecule is an  
2 exogenous small molecule.

1                   7.     The method according to claim 5, wherein said small molecule is a  
2 therapeutic agent or a candidate therapeutic agent.

1                   8.     The method according to claim 7, wherein said small molecule is an  
2 exogenous small molecule.

- 1                   **9.**     The method according to claim 1, wherein said macromolecule is  
2 further labeled with deuterium.
- 1                   **10.**    The method according to claim 1, wherein said biological compartment  
2 is present in a suspension.
- 1                   **11.**    The method according to claim 1, wherein said structural information  
2 is conformational information.
- 1                   **12.**    The method according to claim 1, wherein said structural information  
2 is for a complex formed between said selected macromolecule and a small molecule selected  
3 from therapeutic agents and candidate therapeutic agents.
- 1                   **13.**    The method according to claim 1, wherein said structural information  
2 is for a complex formed between said selected macromolecule and a member selected from  
3 small molecules, endogenous macromolecules and combinations thereof.
- 1                   **14.**    The method according to claim 1, wherein said structural information  
2 is for a first conformation of said selected macromolecule and a second conformation of said  
3 selected macromolecule.
- 1                   **15.**    The method according to claim 1, wherein said data set is acquired by  
2 a triple resonance NMR method.
- 1                   **16.**    The method according to claim 15, wherein said triple resonance NMR  
2 experiment is a member selected from HSQC and TROSY.
- 1                   **17.**    The method according to claim 1, wherein said biological compartment  
2 is prepared by a method comprising:  
3                   (a) transforming an unlabeled precursor of said labeled biological compartment with  
4                   a nucleic acid encoding said selected macromolecule, wherein said nucleic  
5                   acid is operably linked to a promoter non-native to said unlabeled precursor  
6                   cell, thereby producing a transformed biological compartment;  
7                   (b) incubating said transformed biological compartment in a medium comprising said  
8                   NMR-detectable nucleus; and

9 (c) inducing said transformed biological compartment, thereby preparing said labeled  
10 biological compartment.

1 18. The method according to claim 17, further comprising:  
2 (d) inhibiting essentially all transcription in said transformed biological compartment,  
3 which is under control of promoters native to said unlabeled precursor  
4 biological compartment, while allowing transcription under control of said  
5 non-native promoter to proceed.

1 19. The method according to claim 17, wherein said medium comprises an  
2 amino acid labeled with said NMR sensitive nucleus.

1 20. The method according to claim 17, wherein said medium is deuterated.

1 21. The method according to claim 17, wherein said biological  
2 compartment is a bacterial cell.

1 22. The method according to claim 17, wherein the non-native promoter  
2 encodes an RNA polymerase that is operable during step (d).

1 23. The method according to claim 17, wherein the non-native promoter is  
2 a phage promoter.

1 24. The method according to claim 18, wherein said inhibiting is caused by  
2 administering an inhibitor to said biological compartment in an amount sufficient to cause  
3 said inhibiting.

1 25. The method according to claim 24, wherein said inhibitor is rifampicin.

1 26. The method of claim 1, wherein said selected macromolecule  
2 experiences a local viscosity at least 2 fold greater than the viscosity of pure water, wherein  
3 said local viscosity and said viscosity of said pure water are determined at the same  
4 temperature.

1 27. The method of claim 1, wherein said selected macromolecule is  
2 present in said biological compartment at a weight percent of up to 0.3% compared to the  
3 total weight of said biological compartment.

- 1                   **28.**     The method of claim 1, wherein said selected macromolecule is  
2 present in said biological compartment at a weight percent of up to 50% compared to the total  
3 weight of said biological compartment.
- 1                   **29.**     The method of claim 1, wherein said selected macromolecule has a  
2 molecular weight of at least 5 kDa.
- 1                   **30.**     The method of claim 1, wherein said selected macromolecule has a  
2 molecular weight of at least 25 kDa.
- 1                   **31.**     The method of claim 1, wherein said selected macromolecule has a  
2 molecular weight of at least 70 kDa.
- 1                   **32.**     The method of claim 1, wherein said biological compartment is a  
2 living cell.
- 1                   **33.**     The method of claim 1, wherein said biological compartment is a cell  
2 that has been metabolically arrested.
- 1                   **34.**     The method of claim 1, wherein said selected macromolecule is  
2 expressed from a plasmid.
- 1                   **35.**     The method of claim 1, using a multidimensional multinuclear method.
- 1                   **36.**     The method of claim 35, using an HNCA experiment.
- 1                   **37.**     The method of claim 35, using an HMQC experiment.
- 1                   **38.**     The method of claim 1, wherein said compartment is a biological cell.
- 1                   **39.**     The method of claim 38, wherein said cell is a prokaryotic cell.
- 1                   **40.**     The method of claim 39, wherein said cell is a *E. coli* cell.
- 1                   **41.**     The method of claim 38, wherein said cell is a eukaryotic cell.
- 1                   **42.**     The method of claim 41, wherein said cell is a yeast cell.
- 1                   **43.**     The method of claim 41, wherein said cell is a mammalian cell.

1 44. The method of claim 43, wherein said cell is a human cell.

1 45. A method of extracting structural information from a NMR data set for  
2 a selected macromolecule of an intact biological compartment wherein said selected  
3 macromolecule is labeled with a NMR-detectable nucleus, such that said nucleus is present in  
4 said macromolecule in an amount greater than is naturally abundant in said macromolecule,  
5 wherein said nucleus is not  $^{19}\text{F}$ , said method comprising:

- 6 (a) contacting said biological compartment with radio frequency energy,
- 7 thereby producing an excited NMR-detectable nucleus, and
- 8 (b) collecting radio frequency data from said excited NMR-detectable
- 9 nucleus, thereby producing said NMR data set.

1 46. The method according to claim 45, wherein said selected  
2 macromolecule is overexpressed in said biological compartment.

1 47. The method according to claim 45, wherein said NMR-detectable  
2 nucleus is present in an amount detectable by NMR of said intact, biological compartment.

1 48. The method according to claim 45, wherein said selected  
2 macromolecule is a member selected from the group consisting of proteins, saccharides,  
3 glycoproteins, and nucleic acids.

1 49. The method according to claim 45, wherein said selected  
2 macromolecule is in a complex with a small molecule.

1 50. The method according to claim 49, wherein said small molecule is an  
2 exogenous small molecule.

1 51. The method according to claim 49, wherein said small molecule is a  
2 therapeutic agent or a candidate therapeutic agent.

1 52. The method according to claim 51, wherein said small molecule is an  
2 exogenous small molecule.

1 53. The method according to claim 45, wherein said macromolecule is  
2 further labeled with deuterium.

1                   **54.**    The method according to claim 45, wherein said biological  
2 compartment is present in a suspension.

1                   **55.**    The method according to claim 45, wherein said structural information  
2 is conformational information.

1                   **56.**    The method according to claim 45, wherein said structural information  
2 is for a complex formed between said selected macromolecule and a small molecule selected  
3 from therapeutic agents and candidate therapeutic agents.

1                   **57.**    The method according to claim 45, wherein said structural information  
2 is for a complex formed between said selected macromolecule and a member selected from  
3 small molecules, endogenous macromolecules and combinations thereof.

1                   **58.**    The method according to claim 45, wherein said structural information  
2 is for a first conformation of said selected macromolecule and a second conformation of said  
3 selected macromolecule.

1                   **59.**    The method according to claim 45, wherein said data set is acquired by  
2 a triple resonance NMR method.

1                   **60.**    The method according to claim 59, wherein said triple resonance NMR  
2 experiment is a member selected from HSQC and TROSY.

1                   **61.**    The method according to claim 45, wherein said biological  
2 compartment is prepared by a method comprising:

- 3                   (a) transforming an unlabeled precursor of said labeled biological compartment with  
4                   a nucleic acid encoding said selected macromolecule, wherein said nucleic  
5                   acid is operably linked to a promoter non-native to said unlabeled precursor  
6                   biological compartment, thereby producing a transformed biological  
7                   compartment;  
8                   (b) incubating said transformed biological compartment in a medium comprising said  
9                   NMR-detectable nucleus; and  
10                  (c) inducing said transformed biological compartment, thereby preparing said labeled  
11                  biological compartment.

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1                   **62.**    The method according to claim 61, further comprising:  
2                   (d) inhibiting essentially all transcription in said transformed biological compartment,  
3                   which is under control of promoters native to said unlabeled precursor  
4                   biological compartment, while allowing transcription under control of said  
5                   non-native promoter to proceed.

1                   **63.**    The method according to claim 61, wherein said medium comprises an  
2                   amino acid labeled with said NMR sensitive nucleus.

1                   **64.**    The method according to claim 61, wherein said medium is deuterated.

1                   **65.**    The method according to claim 61, wherein said biological  
2                   compartment is a bacterial cell.

1                   **66.**    The method according to claim 61, wherein the non-native promoter  
2                   encodes an RNA polymerase that is operable during step (d).

1                   **67.**    The method according to claim 61, wherein the non-native promoter is  
2                   a phage promoter.

1                   **68.**    The method according to claim 62, wherein said inhibiting is caused by  
2                   administering an inhibitor to said biological compartment in an amount sufficient to cause  
3                   said inhibiting.

1                   **69.**    The method according to claim 68, wherein said inhibitor is rifampicin.

1                   **70.**    The method of claim 45, wherein said selected macromolecule  
2                   experiences a local viscosity at least 2 fold greater than the viscosity of pure water, wherein  
3                   said local viscosity and said viscosity of said pure water are determined at the same  
4                   temperature.

1                   **71.**    The method of claim 45, wherein said selected macromolecule is  
2                   present in said biological compartment at a weight percent of up to 0.3% compared to the  
3                   total weight of said biological compartment.

1                   72.     The method of claim 45, wherein said selected macromolecule is  
2 present in said biological compartment at a weight percent of up to 50% compared to the total  
3 weight of said biological compartment.

1                   73.     The method of claim 45, wherein said selected macromolecule has a  
2 molecular weight of at least 5 kDa.

1                   74.     The method of claim 45, wherein said selected macromolecule has a  
2 molecular weight of at least 25 kDa.

1                   75.     The method of claim 45, wherein said selected macromolecule has a  
2 molecular weight of at least 70 kDa.

1                   76.     The method of claim 45, wherein said biological compartment is a  
2 living cell.

1                   77.     The method of claim 45, wherein said biological compartment is a cell  
2 that has been metabolically arrested.

1                   78.     The method of claim 45, wherein said selected macromolecule is  
2 expressed from a plasmid.

1                   79.     The method of claim 45, using a multidimensional multinuclear  
2 method.

1                   80.     The method of claim 79, using an HNCA experiment.

1                   81.     The method of claim 79, using an HMQC experiment.

1                   82.     The method of claim 45, wherein said compartment is a biological cell.

1                   83.     The method of claim 82, wherein said cell is a prokaryotic cell.

1                   84.     The method of claim 83, wherein said cell is a *E. coli* cell.

1                   85.     The method of claim 83, wherein said cell is a eukaryotic cell.

1                   86.     The method of claim 85, wherein said cell is a yeast cell.



- 1           **87.**    The method of claim **85**, wherein said e cell is a mammalian cell.
- 1           **88.**    The method of claim **87**, wherein said cell is a human cell.

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